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(54) Title: NOCATHIACIN ANTIBIOTIC DERIVATIVES PREPARED BY MICROBIAL BIOTRANSFORMATION


(57) Abstract

Fermentation of Actinoplanes sp. ATCC 53771 or Amycolata autotrophica ATCC 35204 in the presence of nocathiacin yields a new compound nocathiacin 6-deoxyglycoside which has broad spectrum antibiotic activity against Gram-positive bacteria and has *in vivo* efficacy in animals.

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# NOCATHIACIN ANTIBIOTIC DERIVATIVES PREPARED BY MICROBIAL BIOTRANSFORMATION

## BACKGROUND OF THE INVENTION

Multidrug-resistant strains of many clinically important pathogenic bacteria, including methicillin-resistant *Staphylococcus aureus* (MRSA), *Streptococcus pneumoniae* , *Mycobacterium tuberculosis* , and *Enterococci* strains are becoming a worldwide health problem. There is an urgent need to discover new agents to treat patients infected with multidrug-resistant bacteria. A new group of thiazolyl peptide antibiotics, (designated herein as nocathiacins) having inhibitory activity at the nanomolar level against Gram-positive bacteria has been discovered. The present invention relates to a novel antibiotic compound, nocathiacin 6-deoxyglucoside, and comprising a process for preparing it by fermentation of nocathiacin I with Actinoplanes sp. ATCC 53771 or Amycolata autotrophica ATCC 35204 or mutants thereof. The nocathiacin biotransformation product described herein exhibits potent antimicrobial activity against Gram-positive bacteria *in vitro*, and exhibits *in vivo* efficacy in a systemic *Staph. aureus* infection model in animals. Nocathiacin 6-deoxyglucoside and its precursor nocathiacin I are antibiotics useful in the treatment of bacterial infections in humans.

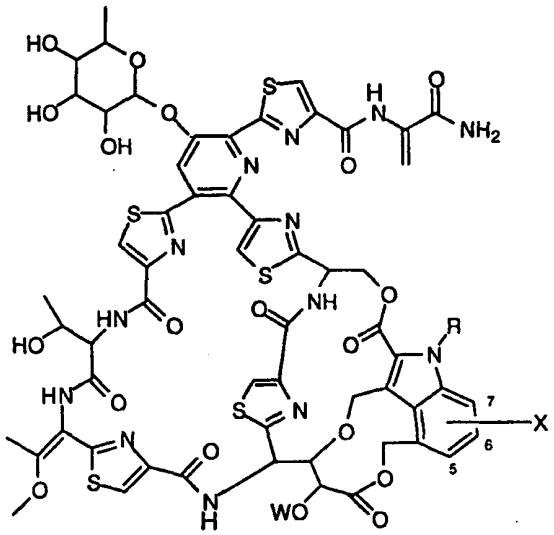
## PRIOR ART

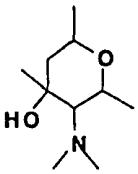
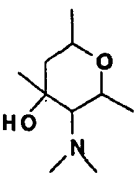
The nocathiacin antibiotics of this invention are derived from nocathiacin I, a novel fermentation product previously described by J. E. Leet *et al* (U. S. Provisional Patent Application Serial No. 60/093,021 filed July 16, 1998) and Sasaki, T. et al, *J. of Antibiotics* 51, No. 8, pp. 715-721 (1998). The nocathiacin antibiotics are related to but clearly distinguishable from nosiheptide (Prange T. *et al., J. Am Chem Soc.* 99, 6418 (1977); Benazet, F. et. al. *Experientia* 36, 414 (1980); Floss, H. G. *et al., J.*

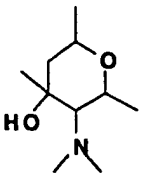
*Am Chem Soc.* **115**, 7557 (1993); α-glycothiohexides (Steinberg, D.A. et al, *J. Antibiot.* **47**, 881 (1994); M. D. Lee et al, *J. Antibiot.* **47**, 881 (1994); M. D. Lee et al, *J. Antibiot.* **47**, 901 (1994); U. S. Patent No. 5,451,581, 1995), and Antibiotic S-54832A (U. S. Patent No. 4,478,831, 1984).

## SUMMARY OF THE INVENTION

The invention concerns novel derivatives of nocathiacin antibiotic I, and others produced by biotransformation and having the structural formula below:



wherein W is  and R is OH, X is H (for nocathiacin I 6-deoxyglycoside); or W is  and R is H, X is H (for nocathiacin II 6-deoxyglycoside); W is H and R is OH, X is H (for nocathiacin III 6-

deoxyglycoside); W is  and R is OH, X is F for fluoronocathiacin I 6-deoxyglycoside.

The nocathiacin derivatives are obtained by the fermentation of Actinoplanes sp. ATCC 53771 or Amycolata autotrophica ATCC 35204 or mutants thereof, in the presence of substrate nocathiacin I. The biotransformation process is accomplished under submerged aerobic conditions in an aqueous medium containing carbon and nitrogen nutrient at a pH of about 5-8 for a sufficient time to produce nocathiacin 6-deoxyglycoside. The resulting analogs exhibit antibiotic activity against a broad spectrum of Gram-positive bacteria, and have improved solubility in aqueous solutions compared to nocathiacin I. Similarly, analogs of nocathiacin II and III are prepared.

The invention also deals with pharmaceutical compositions and methods for treating bacterial infections with the nocathiacin antibiotic derivatives, as well as a biologically pure culture of Actinoplanes sp. ATCC 53771 or Amycolata autotreophica ATCC 35204 from which the antibiotic is obtained. The invention includes all pharmaceutically acceptable derivatives of the nocathiacin 6-deoxyglycoside antibiotics, such as the salts and esters thereof.

The utility of the subject compounds in the treatment of bacterial infections is based upon the expectation that compounds which inhibit Gram-positive bacteria *in vitro* and *in vivo* can be used as antibiotics in animals, and in particular, humans. The compounds of this invention were found to have antibiotic activity, particularly in inhibiting the growth of Gram-positive bacteria.

## BRIEF DESCRIPTION OF THE FIGURES

Figure 1: UV spectrum of nocathiacin 6-deoxyglycoside

Figure 2: IR spectrum of nocathiacin 6-deoxyglycoside

Figure 3: $^{1}$H-NMR spectrum (500 MHz) of nocathiacin 6-deoxyglycoside in deuterated dimethylsulfoxide

Figure 4: $^{13}$C-NMR (125 MHz) spectrum of nocathiacin 6-deoxyglycoside in deuterated dimethylsulfoxide

Figure 5: UV spectrum of nocathiacin II 6-deoxyglycoside

Figure 6: IR spectrum of nocathiacin II 6-deoxyglycoside

Figure 7: $^{1}$H-NMR spectrum (500 MHz) of nocathiacin II 6-deoxyglycoside in deuterated dimethylsulfoxide

Figure 8: UV spectrum of nocathiacin III 6-deoxyglycoside

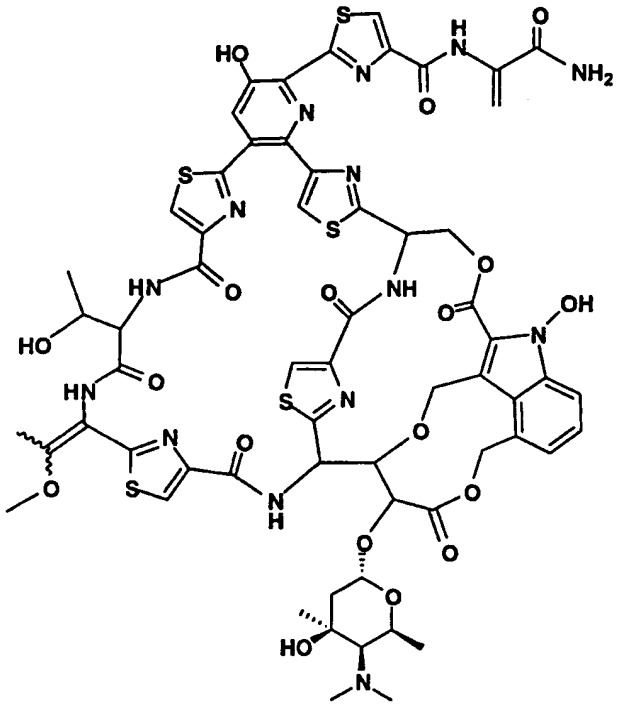
Figure 9: UV spectrum of 5-fluoronocathiacin I 6-deoxyglycoside

## DESCRIPTION OF THE INVENTION

The present invention describes novel derivative of nocathiacin antibiotics obtained through microbial biotransformation. The invention provides an efficient method for the preparation of nocathiacin 6-deoxyglycoside from nocathiacin. The novel process of this invention comprises fermentation of Actinoplanes sp. ATCC 53771, Amycolata autotrophica ATCC 35204, or mutants thereof in the presence of substrate nocathiacin I in a nutrient medium, and isolation of the resulting

biotransformation product, nocathiacin 6-deoxyglycoside, in a conventional manner.

Nocathiacin I has the structural formula below (i.e., W is the glycoside instead of H; R is OH; and X is H). Nocathiacin II is the same as I, except R is H. In Nocathiacin III, W is H; R is OH; and X is H.



The microorganisms, Actinoplanes sp. ATCC 53771 and Amycolata autotrophica ATCC 35204, employed in the present invention may be any microorganism capable of converting nocathiacin to nocathiacin 6-deoxyglycoside. The microorganism, regardless of origin or purity, may be employed in the free state or immobilized on a support such as by physical adsorption or entrapment. The preferred biotransformation microorganisms that were used in this study for conversion of

nocathiacin to nocathiacin 6-deoxyglucoside were obtained from American Type Culture Collection. The taxonomic analysis of Actinoplanes sp. ATCC 53771 has been described in U.S. Patent 4,981,792 (January 1, 1991). The taxonomic analysis of Amycolata autotrophica ATCC 35204 has been described in J. Antibiotics 36: 1176-1183, 1983.

In general, nocathiacin 6-deoxyglycoside can be produced by culturing the aforementioned microorganism in the presence of an appropriate concentration of substrate nocathiacin in an aqueous nutrient medium containing sources of assimilable carbon and nitrogen, preferably under submerged aerobic conditions.

The aqueous medium is incubated at a temperature between 26°C and 32°C, preferably at 28°C. The aqueous medium is incubated for a period of time necessary to complete the biotransformation as monitored by high pressure liquid chromatography (HPLC) usually for a period of about 20-48 hours after the addition of the substrate, on a rotary shaker operating at about 250 rpm with a throw of about 2 inches.

Growth of the microorganisms may be achieved by one of ordinary skill of the art by the use of appropriate medium. Appropriate media for growing microorganism include those which provide nutrients necessary for the growth of microbial cells. A typical medium for growth includes necessary carbon sources, nitrogen sources, and trace elements. Inducers may also be added. The term inducer as used herein, includes any compound enhancing formation of the desired enzymatic activity within the microbial cell.

Carbon sources may include sugars such as glucose, fructose, galactose, maltose, sucrose, mannitol, sorbital, glycerol starch and the like;

organic acids such as sodium acetate, sodium citrate, and the like; and alcohols such as ethanol, propanol and the like.

Nitrogen sources may include N-Z amine A, corn steep liquor, soybean meal, beef extract, yeast extract, tryptone, peptone, cottonseed meal, peanut meal, amino acids such as sodium glutamate and the like, sodium nitrate, ammonium sulfate and the like.

Trace elements may include magnesium, manganese, calcium, cobalt, nickel, iron, sodium and potassium salts. Phosphates may also be added in trace or preferably, greater than trace amounts.

The medium employed may include more than one carbon or nitrogen source or other nutrient. Preferred media for growth include aqueous media, particularly that described in the example herein.

The product, nocathiacin 6-deoxyglucoside, can be recovered from the culture medium by conventional means which are commonly used for the recovery of other known biologically active substances. Accordingly nocathiacin 6-deoxyglycoside can be obtained upon extraction of the culture with a conventional solvent, such as ethyl acetate, treatment with a conventional resin (e.g. anion or cation exchange resin, non-ionic adsorption resin), treatment with a conventional adsorbent (e.g. activated charcoal, silica gel, cellulose, alumina), crystallization, recrystallization, and/or purification by reverse phase preparative HPLC.

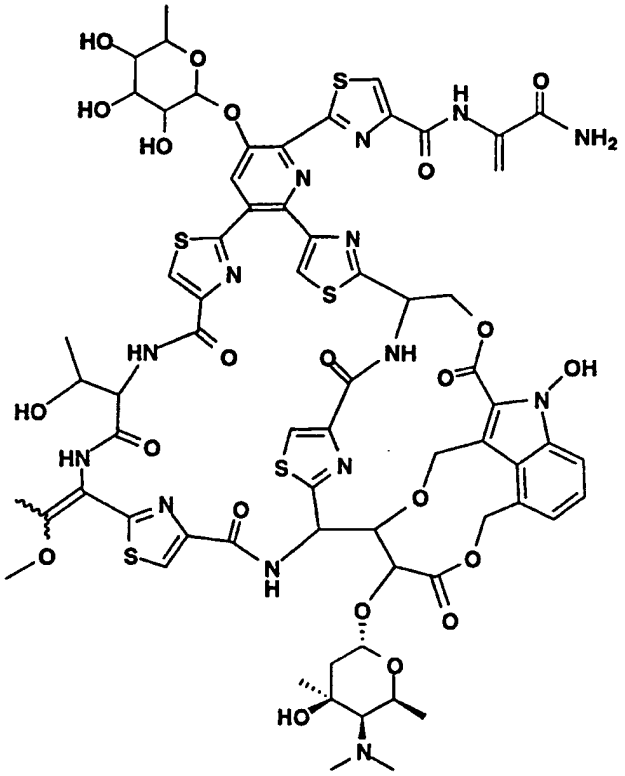
The following examples set out the preparation of nocathiacin antibiotic derivative and its biological properties. Reasonable variations, such as those which would occur to a skilled artisan can be made herein without departing from the scope of the invention.

Microorganisms and Culture Conditions

Actinoplanes sp. ATCC 53771 or Amycolata autotrophica ATCC 35204 can be used as the biotransformation host to convert nocathiacin to a novel product, nocathiacin 6-deoxyglycoside. The biotransformation process utilizing Actinoplanes sp. ATCC 53771 as the biotransformation host is shown in Example 1 below. The example of using Amycolata autotrophica ATCC 35204 as the biotransformation host for the conversion of nocathiacin to nocathiacin 6-deoxyglycoside is as follows. From the frozen vegetative stock culture of Amycolata autotrophica ATCC 35204, 2 ml was used to inoculate 100 ml of seed medium contained the following per liter of deionized water: Glucose, 10 g; polypeptone, 5 g; yeast extract, 3 g; malt extract, 3 g, in a 500-ml flask. The culture was incubated at 28°C on a rotary shaker operating at 250 rpm for 63 hours. The resulting culture from two flasks was pooled and 3 ml of this culture was used to inoculate each of forty-two 500-ml flasks containing the biotransformation medium which has the same composition as the seed medium. The biotransformation cultures were incubated at 28°C on a rotary shaker operating at 250 rpm for 48 hours. Four hundred and twenty mg nocathiacin in 63 ml DMSO was then distributed equally into the biotransformation cultures (1.5 ml/culture). The cultures were then returned to the shaker and incubated for additional 28 hours at 28°C and 250 rpm. The cultures were then processed for the recovery of the nocathiacin analog.

Isolation and structural characterization

The purification of nocathiacin antibiotic biotransformation products from either Actinoplanes sp. ATCC 53771 or Amycolata autotrophica ATCC 35204 using nocathiacin as a substrate was monitored using C18 HPLC-UV. Extraction with ethyl acetate, followed by solvent partitioning, yielded a complex of nocathiacin biotransformation products. Final purification of the individual components was accomplished by reverse phase (C18) preparative HPLC. Spectral data indicated that the compounds are derivatives of the substrate, nocathiacin. The structure of nocathiacin 6-deoxyglycoside, shown below, was assigned based on 2D NMR studies and positive ion electrospray HRMS and MS/MS data.



Materials:

Hexanes, chloroform, (anhydrous ACS grade), and methanol, acetonitrile (anhydrous HPLC grade) were obtained from the Fisher Scientific Company. These solvents were not repurified or redistilled. Water used in chromatography experiments refers to in-house deionized water passed through a Millipore 4 cartridge reagent grade water system (10 mega ohm Milli-Q water).

Analytical HPLC:

The purification of the nocathiacin biotransformation products was monitored by HPLC analysis on an APEX 5 μ ODS column, 4.6 mm i.d. x 15 cm l. (product of Jones Chromatography Inc., Lakewood, CO). Analyses were done on a Hewlett Packard 1100 Series Liquid Chromatograph, with UV detection at 254 nm. A gradient system of acetonitrile and 0.01M potassium phosphate buffer pH 3.5 was used, according to the method of D.J. Hook et.al. (J. Chromatogr. 385, 99 (1987). The eluant was pumped at a flowrate of 1.2 ml/min.

Preparative HPLC:

The following components were used to construct a preparative HPLC system: Beckman Instruments Inc. (Somerset, NJ), Beckman "System Gold" 126 Programmable Solvent Module; Beckman 166 Programmable Detector Module; Beckman "System Gold" Version 711U software; IBM PS/2 55SX System Controller; Preparative HPLC column (reverse phase: C18; Rainin Dynamax C18, Microsorb 8μ, 21.4 mm i.d. x 25 cm l column module; 21.4 mm x 5 cm l. guard module); mobile phase 0.1M ammonium acetate - acetonitrile 55:45 v/v with a linear gradient to

acetonitrile over 30 minutes. Alternative column: YMC Inc. (Wilmington, NC) ODS-AQ 5μ particle size, 120 Å pore size, 20 mm i.d. x 150 mm l., fitted with a ODS-A 25μ particle size, 120 Å pore size, 10 m m i.d. x 10 mm l. drop-in guard module); flow rate 10 ml/min. UV detection: 290 nm.

Analytical Instrumentation

Low resolution MS measurements were performed with a Finnigan SSQ 7000 single quadrupole mass spectrometer, using the positive electrospray ionization mode. MS/MS measurements were conducted in the positive electrospray ionization mode with a Finnigan TSQ 7000 tandem quadrupole mass spectrometer using Argon collision gas or a Finnigan LCQ ion trap mass spectrometer. High resolution MS data were determined with a Finnigan MAT 900 magnetic sector mass spectrometer, positive electrospray ionization mode, ppg reference. The UV spectra were obtained using a Hewlett-Packard 8452A diode array spectrophotometer. IR measurements were taken on a Perkin Elmer 2000 Fourier Transform spectrometer. $^{1}$H-NMR and $^{13}$C-NMR spectra were obtained on a Bruker DRX-500 instrument operating at 500.13 and 125.76 MHz, respectively, using a Nalorac microprobe. Chemical shifts are reported in ppm relative to solvent (DMSO-$d_6$, $\delta_H$ 2.49; $\delta_C$ 39.6).

When the nocathiacin compounds herein are employed as pharmaceutical compositions for the treatment of bacterial infections, they may be combined with one or more pharmaceutically acceptable carriers, for example, solvents, diluents and the like, and may be administered orally in such forms as tablets, capsules, dispersible powders, granules, or suspensions containing, for example, from about 0.05 to 5% of suspending agent, syrups containing, for example, from about 10 to 50% of sugar, and elixirs containing, for example, from about 20 to 50% ethanol,

and the like, or parenterally in the form of sterile injectable solutions or suspension containing from about 0.05 to 5% suspending agent in an isotonic medium. Such pharmaceutical preparations may contain, for example, from about 0.05 up to about 90% of the active ingredient in combination with the carrier, more usually between about 5% and 60% by weight.

The effective dosage of active ingredient employed may vary depending on the particular compound employed, the mode of administration and the severity of the condition being treated. However, in general, satisfactory results are obtained when the compounds of the invention are administered at a daily dosage of from about 0.5 to about 500 mg/kg of animal body weight, preferably given in divided doses two to four times a day, or in sustained release form. For most large mammals the total daily dosage is from about 1 to 100 mg, preferably from about 2 to 80 mg. dosage forms suitable for internal use comprise from about 0.5 to 500 mg of the active compound in intimate admixture with a solid or liquid pharmaceutically acceptable carrier. This dosage regimen may be adjusted to provide the optimal therapeutic response. For example, several divided doses may be administered daily or the dose may be proportionally reduced as indicated by the exigencies of the therapeutic situation.

These active compounds may be administered orally as well as by intravenous, intramuscular, or subcutaneous routes. Solid carriers include starch, lactose, dicalcium phosphate, microcrystalline cellulose, sucrose and kaolin, while liquid carriers include sterile water, polyethylene glycols, non-ionic surfactants and edible oils such as corn, peanut and sesame oils, as are appropriate to the nature of the active ingredient and the particular form of administration desired. Adjuvants customarily employed in the preparation of pharmaceutical compositions

may be advantageously included, such as flavoring agents, coloring agents, preserving agents, and antioxidants, for example, vitamin E, ascorbic acid, BHT and BHA.

These active compounds may also be administered parenterally or intraperitoneally. Solutions or suspensions of these active compounds as a free base or pharmacologically acceptable salt can be prepared in water suitably mixed with a surfactant such as hydroxypropylcellulose. Dispersions can also be prepared in glycerol, liquid polyethylene glycols and mixtures thereof in oils. Under ordinary conditions of storage and use, these preparations contain a preservative to prevent the growth of microorganisms.

The pharmaceutical forms suitable for injectable use include sterile aqueous solutions or dispersions. In all cases, the form must be sterile and must be fluid to the extent that easy syringability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (e.g., glycerol, propylene glycol and liquid polyethylene glycol), suitable mixtures thereof, and vegetable oils.

The term pharmaceutically acceptable salt includes solvates, hydrates, acid addition salts and quaternary salts. The acid addition salts are formed from a nocathiacin compound having a basic nitrogen and a pharmaceutically acceptable inorganic or organic acid including but not limited to hydrochloric, hydrobromic, sulfuric, phosphoric, methanesulfonic, acetic, citric, malonic, succinic, fumeric, maleic, sulfamic, or tartaric acids. Quaternary salts are formed from a basic

nocathiacin compound and an alkyl or arylalkyl halide, preferably methyl or benzyl bromide.

The term "halogen" refers to bromine, chlorine, fluorine or iodine.

## EXAMPLES

The following examples set out the preparation of nocathiacin antibiotic microbial transformation products and their biological properties. Reasonable variations, such as those which would occur to a skilled artisan can be made herein without departing from the scope of the invention.

## NOCATHIACIN BIOTRANSFORMATION PRODUCTS

### EXAMPLE 1: Nocathiacin 6-deoxyglucoside

Reaction Culture:

From the frozen vegetative stock culture of Actinoplanes sp. ATCC 53771, 0.5 ml was used to inoculate 100 ml of seed medium contained the following per liter of deionized water: dextrin, 10 g; glucose, 1 g; beef extract, 3 g; Aradamine pH, 5 g; NZ Amine Type E, 5 g; potassium phosphate, 0.37 g; calcium carbonate, 0.5 g; magnesium sulfate, 0.05 g, in a 500-ml flask. The culture was incubated at 28°C on a rotary shaker operating at 250 rpm for 47 hours. The resulting culture from four flasks was pooled and 10 ml of this culture was used to inoculate each of the thirty 500-ml flasks containing the biotransformation medium with the following per liter of deionized water: Glucose, 10 g; HY-case SF, 2 g; beef extract, 1 g; corn steep liquor, 3 g. The biotransformation cultures were incubated at 28°C on a rotary shaker operating at 250 rpm for 23 hours.

Three hundred mg nocathiacin I in 45 ml DMSO was then distributed equally into the biotransformation cultures (1.5 ml per culture). The cultures were then returned to the shaker and incubated for additional 48 hours at 28°C and 250 rpm. The cultures were then processed for the recovery of the nocathiacin analog.

Preparation of Crude Extract:

Fermentation broth of Actinoplanes sp. ATCC 53771 (3 L.) was extracted (whole broth including mycelia) with approximately 1.5 L. ethyl acetate by vigorous shaking. The biphasic mixture was vacuum filtered through a pad of dicalite. The phases were separated and the lower, aqueous portion extracted two additional times with 1.0 L ethyl acetate. The pale yellow ethyl acetate extracts were pooled and evaporated in vacuo to dryness in a rotary evaporator to yield approximately 240 mg of Residue A.

Liquid-Liquid Partition of Residue A:

Residue A (240 mg) was dissolved in 20 ml of 10% water in methanol. The solution was transferred to a separatory funnel and extracted 3 times with an equal volume of hexane. The hexane layer was removed. The aqueous methanol phase was diluted to 35% water in methanol by adding 7.6 ml of water and extracted 3 times with an equal volume of chloroform. The chloroform had been previously saturated with 35% water in methanol. The hexane, chloroform, and aqueous methanol extracts were evaporated to dryness in vacuo in a rotary evaporator. Nocathiacin antibiotics were detected primarily in the chloroform fraction, (Residue B, 204 mg).

Isolation of Nocathiacin 6-deoxyglycoside:

Residue B was further purified using the specified Beckman System Gold preparative HPLC system; Rainin C18 column. A typical sample injection size was 50 mg/100 µl DMSO. Elution was begun with 0.1M ammonium acetate-acetonitrile 55:45 v/v with a 30 minute linear gradient to acetonitrile. Elution flow rate was 10 ml/min. Detection (UV) was at 290 nm. In this manner, nocathiacin 6-deoxyglycoside (14 minute peak, 36 mg total yield) was obtained.

Preparation of Nocathiacin 6-deoxyglycoside hydrochloride salt:

Nocathiacin 6-deoxyglycoside (20.1 mg; 0.0127 mmol) was mixed with 100 µl MeOH and dissolved in 1 ml tetrahydrofuran. To the suspension was added 1 equivalent 0.1N HCl (127 µl). After 5 minutes, the resulting clear solution was evaporated, resuspended in water and lyophilized.

**Physico-Chemical Properties of Nocathiacin 6-Deoxyglucoside (Free Base)**

| | |
|---|---|
| Description: | buff amorphous solid |
| Solubility: | 0.5 mg/ml in 10% DMSO-Water |
| Molecular Formula: | $C_{67}H_{70}N_{14}O_{22}S_5$ |
| Molecular Weight: | 1582 |

| | |
|---|---|
| Mass Spectrum: | HR-ESIMS $[M+H]^+$ m/z 1583.352<br>ESI-MS/MS fragmentation ions: m/z 1437, 1412, 1266, 1248, 1221, 1204, 1186, 788 |
| Infrared Spectrum: | Major IR Bands ($cm^{-1}$) 3383, 2929, 1719, 1662, 1533, 1478, 1434, 1384, 1319, 1251, 1208, 1162, 1129, 1089, 1035, 1000, 952, 886, 806, 746. |
| Ultraviolet Spectrum: | $\lambda_{max}$ (MeOH) nm 225, 290, 350 (log ε 4.97, 4.61, 4.32). |
| Circular Dichroism: | CD λ nm (Δε) (MeOH) 212 (+15.4), 239 (-59.3), 268 (+28.4), 305 (-11.4), 349 (+11.6). |
| HPLC (Rt) | 18.9 min; (C18; Acetonitrile - 0.01M potassium phosphate buffer pH 3.5 gradient (J. Chromatogr. 385, 99 (1987)). |
| $^1$H-NMR | Observed Chemical Shifts (relative to DMSO-$d_6$ signal δ 2.49):<br><br>δ 9.97 (1H, s), 9.08 (1H, s), 8.65 (1H, s), 8.61 (1H, s), 8.59 (1H, d, J=8.4 Hz), 8.51 (1H, s), 8.26 (1H, s), 8.25 (1H, s), 8.21 (1H, s br), 7.93 (1H, s), 7.87 (1H, d, J=10.8 Hz), 7.78 (1H, s br), 7.72 (1H, d, J=8.4 Hz), 7.35 (1H, dd J=7.8, 7.2 Hz), 7.17 (1H, d, J=6.9 Hz), 6.54 (1H, s), 5.99 (1H, d, J=12.2 Hz), 5.95 (1H, s), 5.78 (1H, s), 5.75 (1H, dd, J=11.0, 4.6 Hz), 5.68 (1H, d, J=8.7 Hz), 5.22 (1H, m), 5.03 (1H, d, J=12.4 Hz), 4.93 (1H, d, J=4.3 Hz), 4.76 (1H, d, J=10.3 Hz), 4.53 (1H, d, J=11.1 Hz), 4.30 (1H, d, J=9.7 Hz), 4.25 (1H, m), 4.23 (1H, m), 4.13 (1H, d, J=10.3 Hz), 4.01 (1H, d, J=9.8 Hz), 3.89 (3H, s), 3.87 (1H, m), 3.76 (1H, m), 3.41 (1H, m), 3.38 (1H, m), 2.49 (6H, s), 2.44 |

(1H, m), 2.02 (1H, s br), 1.98 (3H, s), 1.94 (1H, m), 1.78 (1H, d, J=14.0 Hz), 1.41 (3H, s), 1.21 (1H, m), 1.19 (3H, d, J=5.8 Hz), 0.56 (3H, d, J=6.5 Hz).

$^{13}$C-NMR Observed Chemical Shifts (relative to DMSO-$d_6$ signal δ 39.6):

δ 171.8, 168.3, 167.7, 167.4, 165.4, 164.6, 163.8, 163.1, 161.5, 161.1, 160.5, 160.4, 159.0, 158.8, 154.1, 150.6, 149.8, 148.8, 148.7, 145.8, 144.2, 138.5, 135.0, 133.8, 129.2, 128.1, 126.7, 126.4, 126.3, 125.5, 124.0, 123.1, 120.4, 119.4, 112.8, 111.2, 109.6, 102.9, 99.7, 95.1, 79.3, 71.4, 70.7, 70.6, 70.3, 69.9, 68.3, 67.6, 67.4, 66.3, 65.2, 64.4, 63.1, 56.2, 55.4, 50.3, 49.9, 44.4, 40.5, 30.6, 29.1, 18.1, 18.0, 17.8, 13.0.

**Physico-Chemical Properties of Nocathiacin 6-Deoxyglycoside (Hydrochloride Salt)**

| | |
|---|---|
| Description: | buff colored amorphous solid |
| Solubility: | >10.5 mg/ml in water |
| Molecular Formula: | $C_{67}H_{70}N_{14}O_{22}S_5$ -HCl |
| Elemental Analysis: | Found C 46.62, H 4.79, N 10.89, S 8.78, Cl 2.29 |
| KF Moisture | 6.1 |
| Formula Weight: | 1618 |

Infrared Spectrum: Major IR Bands ($cm^{-1}$) 3384, 2929, 1719, 1662, 1533, 1478, 1434, 1384, 1319, 1251, 1208, 1161, 1129, 1088, 1001, 952, 886, 789, 746.

## NOCATHIACIN BIOTRANSFORMATION PRODUCTS

### EXAMPLE 2: Nocathiacin I- 6-deoxyglycoside and Nocathiacin II- 6-deoxyglycoside

Reaction Culture:

From the frozen vegetative stock culture of Actinoplanes sp. ATCC 53771, 2 ml was used to inoculate 100 ml of seed medium containing the following per liter of deionized water: dextrin, 10 g; glucose, 1 g; beef extract, 3 g; Aradamine pH, 5 g; NZ Amine Type E, 5 g; potassium phosphate, 0.37 g; calcium carbonate, 0.5 g; magnesium sulfate, 0.05 g, in a 500-ml flask. The culture was incubated at 28°C on a rotary shaker operating at 250 rpm for 44.5 hours. The resulting culture from eight flasks was pooled and 10 ml of this culture was used to inoculate each of the fifty 500-ml flasks containing the medium with the following per liter of deionized water: Glucose, 10 g; HY-case SF, 2 g; beef extract, 1 g; corn steep liquor, 3 g. The cultures were incubated at 28°C on a rotary shaker operating at 250 rpm for 24.5 hours. The culture was pooled and distributed into six 1 L centrifuge bottles at approximately equal volume. After centrifugation at 3000 rpm for 15 min, the supernatant was discarded and the cell was centrifuge washed with 200 ml sterile water. To each bottle, 350 ml of potassium phosphate buffer (100 mM, pH 6.8) containing 50 g/L dextrose was added and the suspension was transferred to a 2L flask. A 1.36 g Nocathiacin I solution in 120 ml of DMSO was prepared and 20 ml of this solution was added to each flask. Five flasks were incubated at 28°C and 200 rpm for 2 days and the reaction mixture was

then processed for the recovery of the nocathiacin analogs. The sixth flask was incubated at 28°C and 200 rpm for 8.5 hours and an additional 200 mg nocathiacin I in 20 ml DMSO was added. After 18 hours, 12 g dextrose was added and the flask was incubated at 28°C and 250 rpm for 1 day before processing for the recovery of the nocathiacin analogs.

Preparation of Crude Extract:

The above reaction mixture (2.4 L.) was extracted (whole broth including mycelia) with approximately 2.4 L. ethyl acetate by vigorous shaking. The biphasic mixture was vacuum filtered through a pad of dicalite. The phases were separated and the lower, aqueous portion extracted one additional time with 2 L. ethyl acetate. The mycelia-dicalite cake was extracted with 1.6 L. ethyl acetate, followed by 1.5 L. chloroform-methanol. The pale yellow ethyl acetate and chloroform-methanol extracts were pooled, dissolved in 35% water in methanol (300 ml) and extracted twice with equal volumes chloroform. The chloroform had been previously saturated with 35% water in methanol. The chloroform extract was evaporated in vacuo to dryness in a rotary evaporator to yield approximately 3.4 g of Residue C.

Silica Gel Vacuum Liquid Chromatography of Residue C:

The crude extract containing nocathiacin antibiotics (Residue C) was preadsorbed onto 3 g Merck LiChroprep Silica Gel 60 (25-40μ) and applied to a 2.5 x 15 cm fritted filter funnel packed half full with this adsorbent (10 g). Elution using house vacuum was initially with chloroform (150 ml), followed by chloroform-methanol-water mixtures in a step gradient (e.g. $CHCl_3$-MeOH-$H_2O$ 98:2:0.2, 97:3:0.3, 95:5:0.5, 93:7:0.7, 90:10:1 (2x), v/v, 100 ml each. Fractions were consolidated on the basis of silica TLC profiles (chloroform-methanol-water 90:10:1 v/v, long

wavelength UV and ceric sulfate spray). In this manner, nocathiacin 6-deoxyglycosides were detected in the $CHCl_3$-MeOH-$H_2O$ 93:7:0.7 and the first $CHCl_3$-MeOH-$H_2O$ 90:10:1 fraction. This fraction was evaporated to dryness, (Residue D, 431 mg).

Isolation of nocathiacin I 6-deoxyglycoside and nocathiacin II 6-deoxyglycoside:

Residue D was further purified using the specified Beckman System Gold preparative HPLC system: YMC-Pack Pro C18 column (5μ particle size, 120 Å pore size, 20 mm i.d. x 150 mm l.), fitted with a ODS-A 25μ particle size, 120 Å pore size, 10 mm i.d. x 10 mm l. drop-in guard module. A typical sample injection size was 65 mg/300 μl DMSO. Elution was begun with 0.1M ammonium acetate-acetonitrile 55:45 v/v with a 30 minute concave gradient to acetonitrile. Elution flow rate was 10 ml/min. Detection (UV) was at 360 nm. In this manner, nocathiacin I 6-deoxyglycoside (14 minute peak, 267 mg total yield) and nocathiacin II 6-deoxyglycoside (6 minute peak, 10 mg total yield) were obtained.

**Physico-Chemical Properties of Nocathiacin II- 6-Deoxyglycoside**

| | |
|---|---|
| Description: | buff amorphous solid |
| Molecular Formula: | $C_{67}H_{70}N_{14}O_{21}S_5$ |
| Molecular Weight: | 1566 |
| Mass Spectrum: | HR-ESIMS $[M+Na]^+$ m/z 1589.329<br>ESI-MS/MS fragmentation ions: m/z 1421, 1396, 1250, 1232, 1206, 1188, 1171, 1139, 788 |

| | |
|---|---|
| Infrared Spectrum: | Major IR Bands ($cm^{-1}$) 3382, 3119, 2934, 1728, 1664, 1533, 1481, 1435, 1385, 1318, 1251, 1193, 1131, 1091, 1076, 1000, 969, 794, 752 |
| Ultraviolet Spectrum: | $\lambda_{max}$ (MeOH) nm 221, 294, 352 (log ε 4.82, 4.47, 4.20). |
| Circular Dichroism: | CD λ nm (Δε) (MeOH) 237 (-33.0), 256 (+12.3), 281sh (+8.9), 306 (-7.9), 350 (+5.2). |
| HPLC (Rt) | 13.2 min; (C18; Acetonitrile - 0.01M potassium phosphate buffer pH 3.5 gradient (J. Chromatogr. 385, 99 (1987)). |
| $^1$H-NMR | Observed Chemical Shifts (relative to DMSO-$d_6$ signal δ 2.49): δ 11.36 (1H, s), 9.96 (1H, s), 9.11 (1H, s), 8.69 (1H, s), 8.66 (1H, s), 8.62 (1H, d, J=8.9 Hz), 8.52 (1H, s), 8.32 (1H, s), 8.24 (1H, s), 7.89 (1H, s), 7.81 (1H, s), 7.70 (1H, d, J=10.8 Hz), 7.60 (1H, d, J=8.3 Hz), 7.27 (1H, dd J=7.7, 7.3 Hz), 7.14 (1H, d, J=6.9 Hz), 7.10 (1H, d, J=8.0 Hz), 6.56 (1H, s), 6.04 (1H, d, J=12.3 Hz), 5.97 (1H, s), 5.79 (1H, s), 5.77 (1H, d br, J=3.4 Hz), 5.69 (1H, d, J=8.5 Hz), 5.26 (1H, m), 5.18 (1H, s br), 5.02 (1H, d, J=12.3 Hz), 4.95 (2H, m), 4.89 (1H, m), 4.71 (1H, d, J=11.3 Hz), 4.56 (1H, m), 4.37 (1H, d, J=9.7 Hz), 4.23 (2H, s br), 4.12 (1H, d, J=10.0 Hz), 4.03 (1H, d, J=9.5 Hz), 3.90 (3H, s), 3.75 (1H, m), 3.56 (1H, m), 3.46 (1H, m), 2.50 (6H, s), 2.00 (3H, s), 1.96 (1H, m), 1.80 (1H, d, J=13.5 Hz), 1.41 (3H, s), 1.25 (1H, m), 1.21 (3H, d, J=5.6 Hz), 0.54 (3H, d, J=6.3 Hz). |

## EXAMPLE 3: Nocathiacin III- 6-deoxyglycoside

Reaction Culture:

From the frozen vegetative stock culture of Actinoplanes sp. ATCC 53771, 2 ml was used to inoculate 100 ml of seed medium containing the following per liter of deionized water: dextrin, 10 g; glucose, 1 g; beef extract, 3 g; Aradamine pH, 5 g; NZ Amine Type E, 5 g; potassium phosphate, 0.37 g; calcium carbonate, 0.5 g; magnesium sulfate, 0.05 g, in a 500-ml flask. The culture was incubated at 28°C on a rotary shaker operating at 250 rpm for 43 hours. The resulting culture from four flasks was pooled and 10 ml of this culture was used to inoculate each of the thirty 500-ml flasks containing the medium with the following per liter of deionized water: Glucose, 10 g; HY-case SF, 2 g; beef extract, 1 g; corn steep liquor, 3 g. The cultures were incubated at 28°C on a rotary shaker operating at 250 rpm for 23 hours. Five of the above flasks were pooled and centrifuged at 3500 rpm for 15 min. After discarding the supernatant, the cell was suspended in potassium phosphate buffer (100 mM, pH 7.0) with 50 g/L dextrose to a final volume of 220 ml in a 500-ml flask. To this mixture, 1.1 ml of 20% $NH_4Cl$ was added. One hundred and eight mg nocathiacin III in 10 ml DMSO was added to the above cell suspension and the reaction mixture was then incubated at 28°C and 200 rpm for 2 days. The reaction mixture was extracted three times each with 200 ml ethyl acetate. The combined ethyl acetate extracts were evaporated to dryness. The residue was then dissolved in 10 ml DMSO and mixed with 250 ml cell suspension as described above. The reaction mixture was distributed approximately in equal volumes into five 500-ml flasks and the flasks were incubated at 28°C and 200 rpm for 2 days. The reaction mixture was then processed for the recovery of the nocathiacin analog.

Preparation of Crude Extract:

The above reaction mixture (250 mL) was extracted (whole broth including mycelia) with approximately 300 mL ethyl acetate (3x) by vigorous shaking. The pale yellow ethyl acetate extracts were pooled and evaporated in vacuo to dryness in a rotary evaporator to yield approximately 100 mg of Residue E.

Isolation of Nocathiacin III 6-deoxyglycoside:

Residue E was further purified using the specified Beckman System Gold preparative HPLC system: YMC-Pack ODS-AQ column (10 μ particle size, 120 Å pore size, 20 mm i.d. x 150 mm l.), fitted with a ODS-A 25μ particle size, 120 Å pore size, 10 mm i.d. x 10 mm l. drop-in guard module. Elution was begun with 0.1M ammonium acetate-acetonitrile 55:45 v/v with a 30 minute linear gradient to acetonitrile. Elution flow rate was 10 ml/min. Detection (UV) was at 290 nm. In this manner, nocathiacin III 6-deoxyglycoside (7 minute peak, 3 mg total yield) was obtained.

**Physico-Chemical Properties of Nocathiacin III 6-Deoxyglycoside**

| | |
|---|---|
| Description: | buff amorphous solid |
| Molecular Formula: | $C_{58}H_{53}N_{13}O_{20}S_5$ |
| Molecular Weight: | 1411 |
| Mass Spectrum: | HR-ESIMS $[M+Na]^+$ m/z 1434.198<br>ESI-MS/MS fragmentation ions: m/z 1266, 1248, 1222, 1204, 1186, 788 |

| | |
|---|---|
| HPLC-UV | Rt 12.0 min; (C18; Acetonitrile - 0.01M potassium phosphate buffer pH 3.5 gradient (J. Chromatogr. 385, 99 (1987)); UV $\lambda_{max}$ 227, 290, 346 nm |

**EXAMPLE 4: 5-Fluoronocathiacin I- 6-deoxyglycoside**

Reaction Culture:

From the frozen vegetative stock culture of Actinoplanes sp. ATCC 53771, 2 ml was used to inoculate 100 ml of seed medium containing the following per liter of deionized water: dextrin, 10 g; glucose, 1 g; beef extract, 3 g; Aradamine pH, 5 g; NZ Amine Type E, 5 g; potassium phosphate, 0.37 g; calcium carbonate, 0.5 g; magnesium sulfate, 0.05 g, in a 500-ml flask. The culture was incubated at 28°C on a rotary shaker operating at 250 rpm for 43 hours. The resulting culture from four flasks was pooled and 10 ml of this culture was used to inoculate each of the thirty 500-ml flasks containing the medium with the following per liter of deionized water: Glucose, 10 g; HY-case SF, 2 g; beef extract, 1 g; corn steep liquor, 3 g. The culture was incubated at 28°C on a rotary shaker operating at 250 rpm for 23 hours. Five of the above flasks were pooled and centrifuged at 3500 rpm for 15 min. After discarding the supernatant, the cell was suspended in potassium phosphate buffer (100 mM, pH 7.0) with 50 g/L dextrose to a final volume of 250 ml in a 500-ml flask. To this mixture, 1.1 ml of 20% $NH_4Cl$ was added. Seventy ml of the above cell suspension was transferred to a 500-ml flask and 32 mg 5-

fluoronocathiacin I in 4 ml DMSO was then added to the above cell suspension. The reaction mixture was then incubated at 28°C and 250 rpm for 40 hours. The reaction mixture was then processed for the recovery of the nocathiacin analog.

Preparation of Crude Extract:

The above reaction mixture (75 mL) was extracted (whole broth including mycelia) with approximately 75 mL ethyl acetate (3x) by vigorous shaking. The pale yellow ethyl acetate extracts were pooled and evaporated in vacuo to dryness in a rotary evaporator to yield approximately 69 mg of Residue F.

Isolation of 5-Fluoronocathiacin I 6-deoxyglycoside:

Residue F was further purified using the specified Beckman System Gold preparative HPLC system: YMC-Pack ODS-AQ column (10 µ particle size, 120 Å pore size, 20 mm i.d. x 150 mm l.), fitted with a ODS-A 25µ particle size, 120 Å pore size, 10 mm i.d. x 10 mm l. drop-in guard module. Elution was begun with 0.1M ammonium acetate-acetonitrile 55:45 v/v with a 30 minute linear gradient to acetonitrile. Elution flow rate was 10 ml/min. Detection (UV) was at 290 nm. In this manner, 5-fluoronocathiacin I 6-deoxyglycoside (10 minute peak, 8 mg total yield) was obtained.

**Physico-Chemical Properties of 5-Fluoronocathiacin I 6-Deoxyglycoside**

| | |
|---|---|
| Description: | buff amorphous solid |
| Molecular Formula: | $C_{67}H_{69}FN_{14}O_{22}S_5$ |
| Molecular Weight: | 1600 |
| Mass Spectrum: | HR-ESIMS $[M+H]^+$ m/z 1601.339<br>ESI-MS/MS fragmentation ions: m/z 1455, 1430, 1284, 1266, 1222, 1204, 1186, 1154, 788 |
| HPLC-UV | Rt 21.1 min; (C18; Acetonitrile - 0.01M potassium phosphate buffer pH 3.5 gradient (J. Chromatogr. 385, 99 (1987)); UV $\lambda_{max}$ 223, 290, 346 nm |

## BIOLOGICAL EVALUATION OF NOCATHIACIN DERIVATIVES

### EXAMPLE 5: Antibiotic Activity of Nocathiacin 6-deoxyglycosides

To demonstrate its antimicrobial properties, the minimum inhibitory concentration (MIC) for nocathiacin antibiotic derivatives of the invention was obtained against a variety of bacteria using a conventional broth dilution assay (serial broth dilution method using nutrient broth (Difco)). The results obtained are shown in Table 1 below, and demonstrate that nocathiacin derivatives have utility in treating bacterial infections.

Table 1.

| Organism | Strain # | Nocathiacin I 6-deoxyglycoside (free base) | Nocathiacin I 6-deoxyglycoside (HCl salt) | Nocathiacin II 6-deoxyglycoside (free base) | Nocathiacin III 6-deoxyglycoside | 5-Fluoro-Nocathiacin I 6-deoxyglycoside (free base) |
|---|---|---|---|---|---|---|
| Streptococcus pneumoniae | A9585 | 0.001 | 0.001 | 0.007 | <0.0005 | <0.0005 |
| Streptococcus pneumoniae/ penicillin intermediate | A27881 | 0.001 | 0.001 | 0.007 | <0.0005 | <0.0005 |
| Streptococcus pneumoniae/ penicillin resistant | A28272 | 0.001 | 0.001 | 0.007 | <0.0005 | <0.0005 |
| Enterococcus faecalis | A20688 | 0.03 | 0.06 | 1 | 0.03 | 0.125 |
| Enterococcus faecalis | A27519 | 0.03 | 0.06 | - | | |
| Enterococcus faecalis +50% calf serum | A20688 | 0.03 | 0.06 | 1 | 0.03 | 0.125 |
| Enterococcus faecium | A24885 | 0.03 | 0.03 | 2 | 0.03 | 0.03 |
| Enterococcus faecium/thios trepton resistant (10 ug/ml) | SC15829 | 0.5 | 0.125 | - | | |
| Enterococcus avium | A27456 | 0.03 | 0.03 | - | | |
| Staphylococcus aureus/β-lactamase positive | A15090 | 0.03 | 0.03 | 0.5 | 0.03 | 0.03 |
| Staphylococcus aureus + 50% calf serum | A15090 | 0.03 | 0.03 | 0.5 | 0.03 | 0.125 |
| Staphylococcus aureus/QC/ ATCC#29213 | A24407 | 0.015 | 0.03 | 2 | 0.03 | 0.03 |
| Staphylococcus aureus /homo methicillin resistant | A27223 | 0.007 | 0.007 | 0.25 | 0.03 | 0.03 |

| | | | | | | |
|---|---|---|---|---|---|---|
| Staphylococcus aureus + 50% calf serum | A27223 | 0.015 | 0.003 | 0.5 | 0.03 | 0.125 |
| Staphylococcus epidermidis | A24548 | 0.03 | 0.015 | 0.5 | 0.015 | 0.125 |
| Staphylococcus haemolyticus | A27298 | 0.03 | 0.03 | 0.5 | 0.004 | 0.125 |
| Moraxella catarrhalis/β-lactamase positive | A22344 | 0.03 | 0.015 | 2 | 0.125 | 0.5 |
| Moraxella catarrhalis/β-lactamase positive | A25409 | 0.03 | 0.015 | 2 | | |

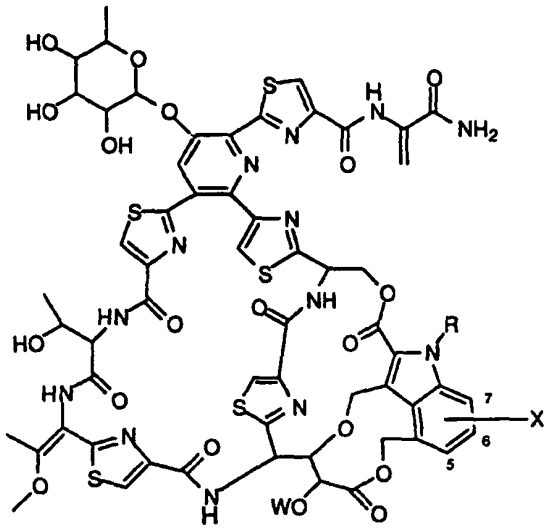
**EXAMPLE 6: Nocathiacin 6-deoxyglycoside *in vivo* Antibiotic Activity in a Systemic *Staph. aureus* Infection Model.**

Nocathiacin 6-deoxyglycoside was evaluated for antibiotic activity *in vivo*, in a systemic infection model using female ICR mice. The animals were infected IP with $6.5 \times 10^6$ CFU of an overnight culture of *Staphylococcus aureus* A15090 suspended in 7% mucin. Nocathiacin 6-deoxyglycoside was dissolved in a test formulation consisting of 10% DMSO, 5% Tween 80 and 85% water. The solution was administered SC at 100 mg/kg total dose (2 x 50 mg/kg doses at 1 and 4 hours post-infection). Nine out of nine animals survived the duration of the experiment with no signs of toxicity. The $PD_{50}$ of nocathiacin 6-deoxyglycoside was determined to be 6.2 mg/kg.

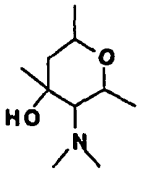
## CLAIMS

We claim:

1. A nocathiacin compound, or a pharmaceutically acceptable salt thereof having the formula



wherein:

W is H or  ;

R is H or OH; and

X is halogen or H.

2. The compound of claim 1, nocathiacin 6-deoxyglycoside antibiotic, or a pharmaceutically acceptable salt thereof, wherein the antibiotic has the following characteristics:

(a) appears as a buff colored amorphous solid;

(b) has a molecular weight of 1582 as determined by mass spectrometry;

(c) has the molecular formula $C_{67}H_{70}N_{14}O_{22}S_5$

(d) exhibits an ultraviolet absorption spectrum when dissolved in methanol substantially as shown in FIG. 1;

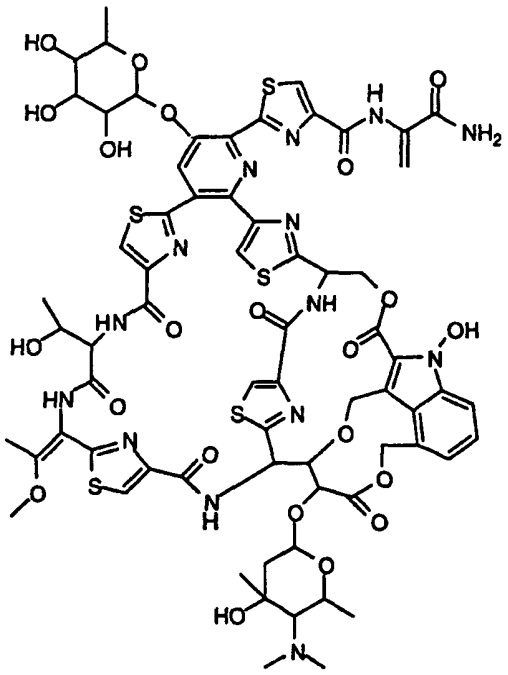
(e) exhibits an infrared absorption spectrum (KBr) substantially as shown in FIG 2;

(f) when dissolved in deuterated dimethylsulfoxide exhibits a proton magnetic resonance spectrum substantially as shown in FIG. 3;

(g) when dissolved in deuterated dimethylsulfoxide exhibits a $^{13}C$ magnetic resonance spectrum substantially as shown in FIG. 4;

(h) exhibits a high performance liquid chromatography retention time of 18.9 minutes with a C18 reversed phase silica gel column using a 0.01M potassium phosphate buffer pH 3.5 - acetonitrile gradient;

(i) and has the formula



3. The compound of claim 2 wherein the pharmaceutically acceptable salt is hydrochloride salt.

4. The compound of claim 1, nocathiacin II 6-deoxyglycoside antibiotic, or a pharmaceutically acceptable salt thereof, wherein the antibiotic has the following characteristics:

(a) appears as a buff colored amorphous solid;

(b) has a molecular weight of 1566 as determined by mass spectrometry;

(c) has the molecular formula $C_{67}H_{70}N_{14}O_{21}S_5$

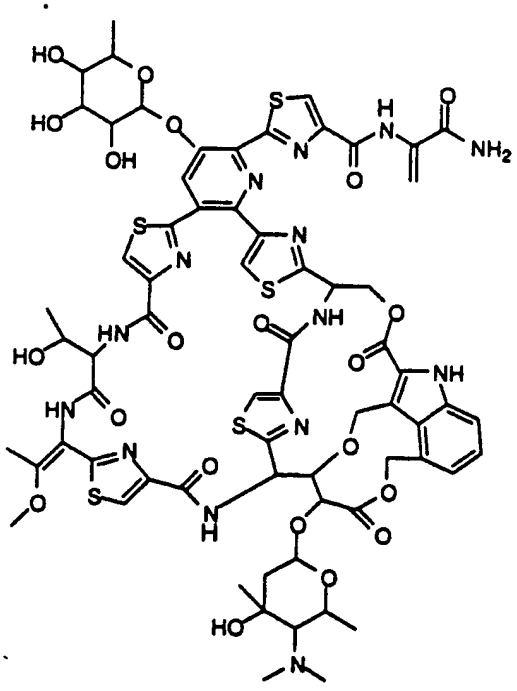
(d) exhibits an ultraviolet absorption spectrum when dissolved in methanol substantially as shown in FIG. 5;

(e) exhibits an infrared absorption spectrum (KBr) substantially as shown in FIG 6;

(f) when dissolved in deuterated dimethylsulfoxide exhibits a proton magnetic resonance spectrum substantially as shown in FIG. 7;

(g) exhibits a high performance liquid chromatography retention time of 13.2 minutes with a C18 reversed phase silica gel column using a 0.01M potassium phosphate buffer pH 3.5 - acetonitrile gradient;

(h) and has the formula



5. The compound of claim 1, nocathiacin III 6-deoxyglycoside antibiotic, or a pharmaceutically acceptable salt thereof, wherein the antibiotic has the following characteristics:

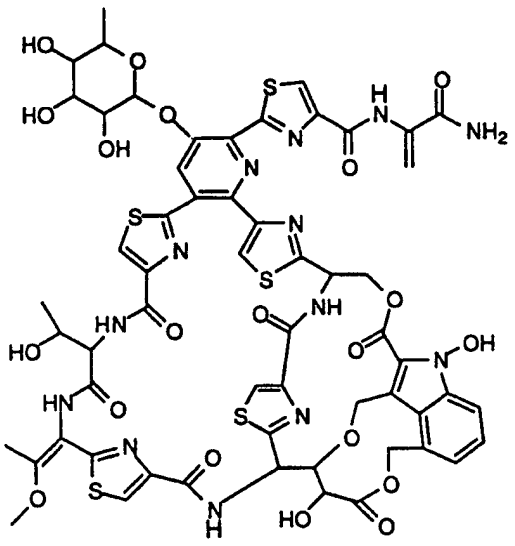
(a) appears as a buff colored amorphous solid;

(b) has a molecular weight of 1411 as determined by mass spectrometry;

(c) has the molecular formula $C_{58}H_{53}N_{13}O_{20}S_5$

(d) exhibits a high performance liquid chromatography retention time of 12.0 minutes with a C18 reversed phase silica gel column using a 0.01M potassium phosphate buffer pH 3.5 - acetonitrile gradient; and exhibits an ultraviolet absorption spectrum upon elution substantially as shown in FIG. 8.

(e) and has the formula



6. The compound of claim 1, 5-fluoronocathiacin I 6-deoxyglycoside antibiotic or a pharmaceutically acceptable salt thereof, wherein the antibiotic has the following characteristics:

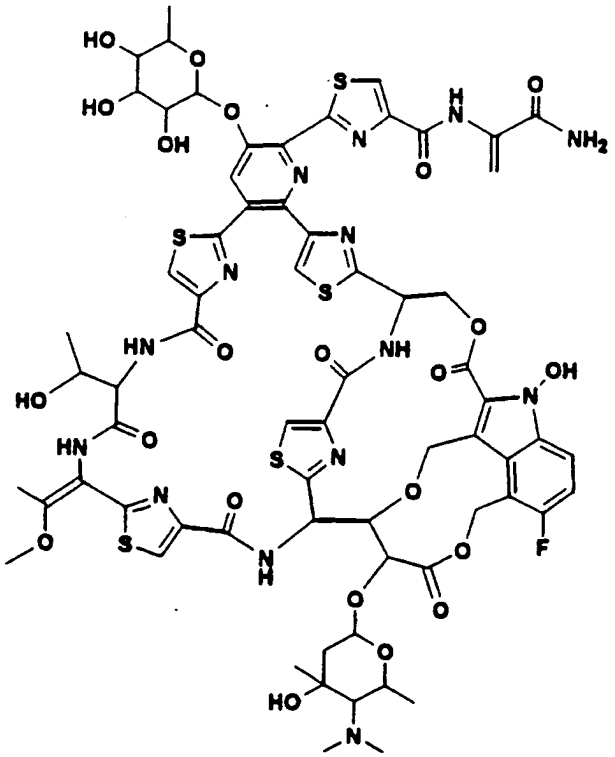
(a) appears as a buff colored amorphous solid;

(b) has a molecular weight of 1600 as determined by mass spectrometry;

(c) has the molecular formula $C_{67}H_{69}FN_{14}O_{22}S_5$

(d) exhibits a high performance liquid chromatography retention time of 21.1 minutes with a C18 reversed phase silica gel column using a 0.01M potassium phosphate buffer pH 3.5 - acetonitrile gradient; and exhibits an ultraviolet absorption spectrum upon elution substantially as shown in FIG. 9.

(e) and has the formula



7. A pharmaceutical composition comprising a therapeutically effective amount of a compound as claimed in any of claims 1 - 6 and a suitable carrier or diluent.

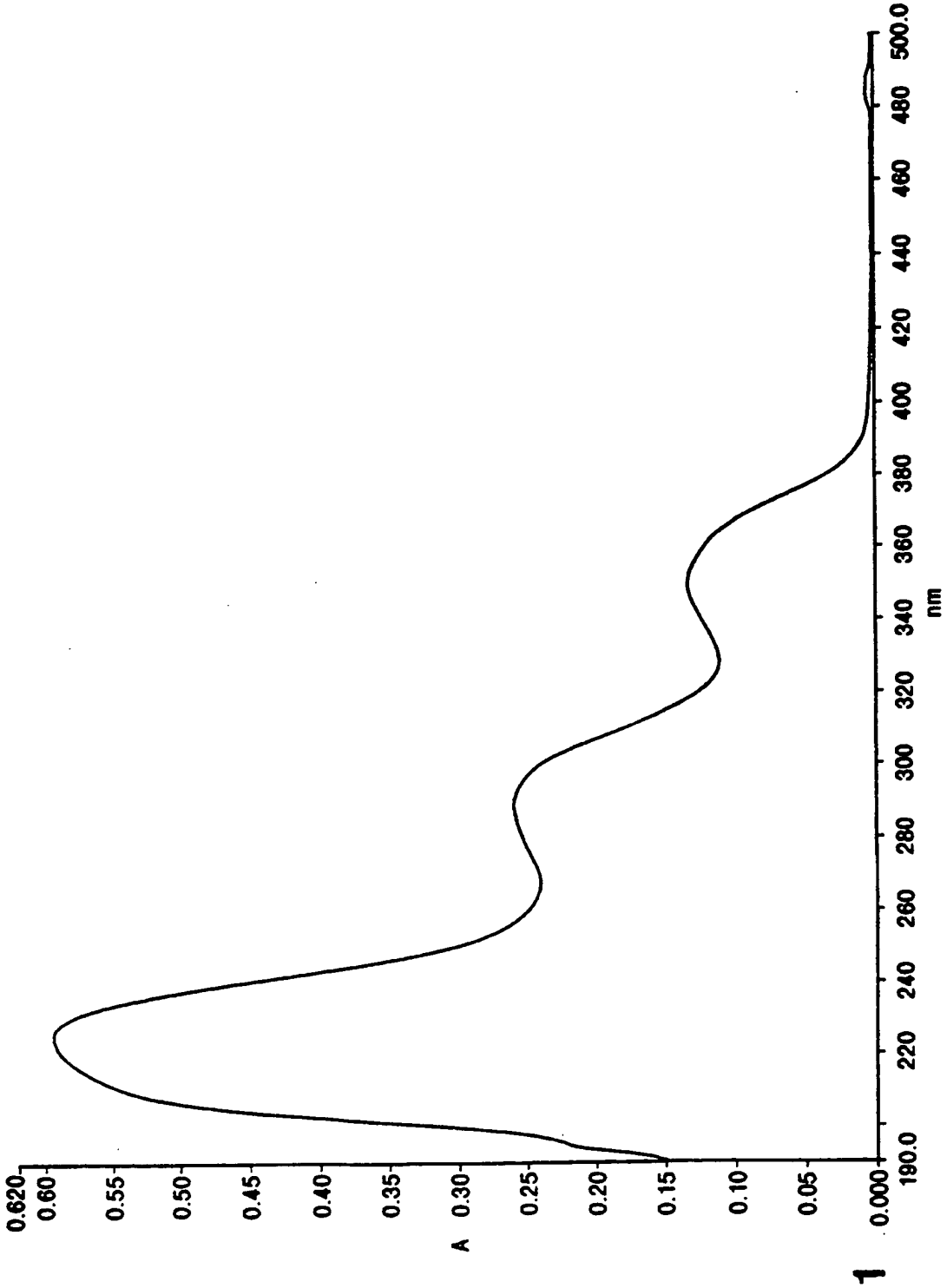
8. A method for preventing or treating infection of a mammal by a bacterium, comprising the step of administering a therapeutically effective amount of a compound as claimed in any of claims 1 -6 to said mammal in need thereof.

9. A method for making nocathiacin I 6-deoxyglycoside from nocathiacin I comprising the steps of converting nocathiacin I to nocathiacin I 6-deoxyglycoside in a biotransformation broth containing fermented culture of microorganism Actinoplanes sp. (ATCC-53771) or Amycolata autotrophica (ATCC-35204), and isolating said nocathiacin I 6-deoxyglycoside from the biotransformation reaction broth.

10. A method for making nocathiacin II 6-deoxyglycoside from nocathiacin II comprising the steps of converting nocathiacin II to nocathiacin II 6-deoxyglycoside in a biotransformation broth containing fermented culture of microorganism Actinoplanes sp. (ATCC-53771) or Amycolata autotrophica (ATCC-35204), and isolating said nocathiacin II 6-deoxyglycoside from the biotransformation reaction broth.

11. A method for making nocathiacin III 6-deoxyglycoside from nocathiacin III comprising the steps of converting nocathiacin III to nocathiacin III 6-deoxyglycoside in a biotransformation broth containing fermented culture of microorganism Actinoplanes sp. (ATCC-53771) or Amycolata autotrophica (ATCC-35204), and isolating said nocathiacin III 6-deoxyglycoside from the biotransformation reaction broth.

12. A method for making 5-fluoronocathiacin I 6-deoxyglycoside from 5-fluoronocathiacin I comprising the steps of converting 5-fluoronocathiacin I to 5-fluoronocathiacin I 6-deoxyglycoside in a biotransformation broth containing fermented culture of microorganism Actinoplanes sp. (ATCC-53771) or Amycolata autotrophica (ATCC-35204), and isolating said 5-fluoronocathiacin I 6-deoxyglycoside from the biotransformation reaction broth.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420
440
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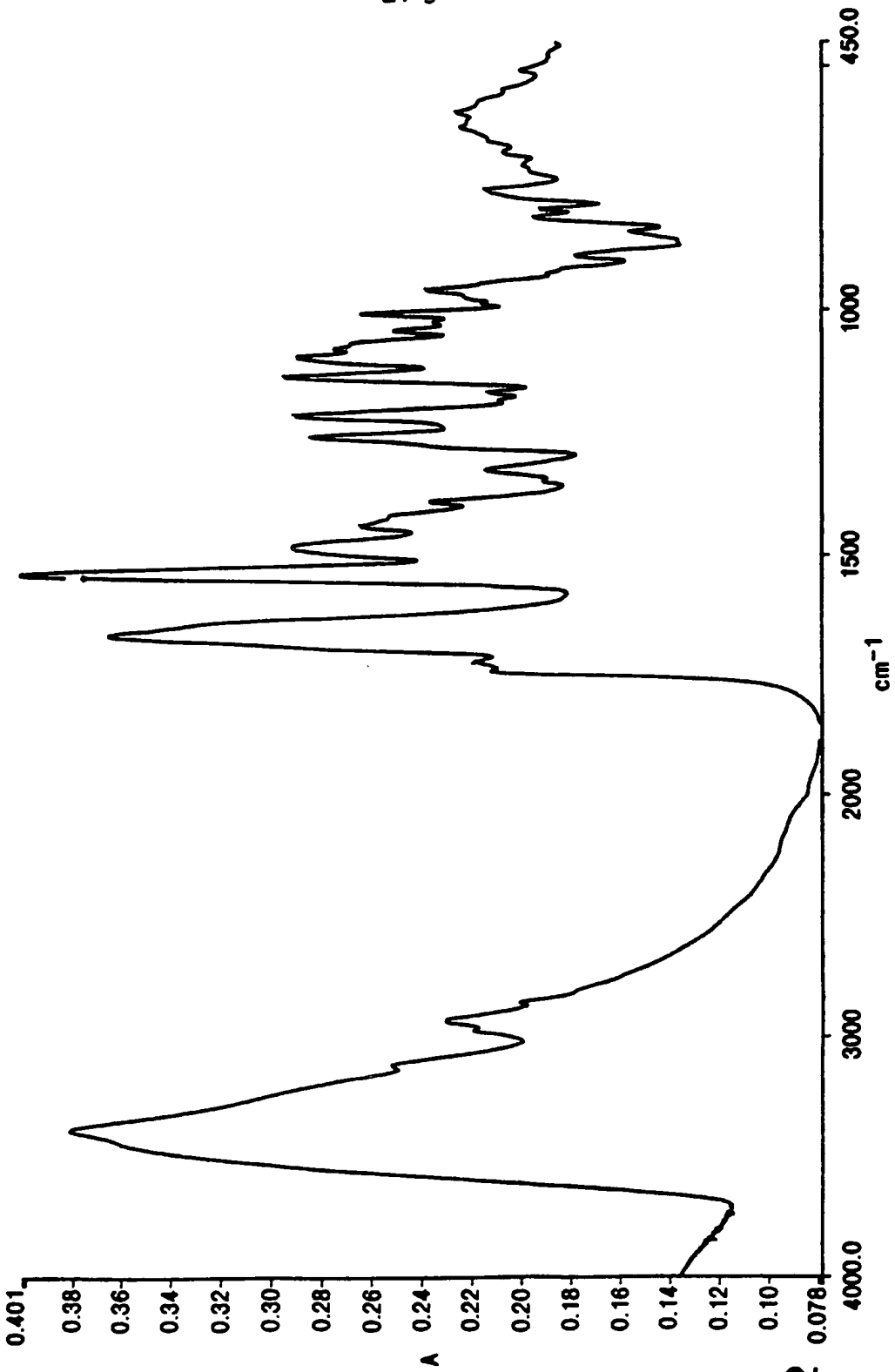
500.0
nm

FIG. 1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0.26
0.24
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0.18
0.16
0.14
0.12
0.10
0.078

FIG. 2

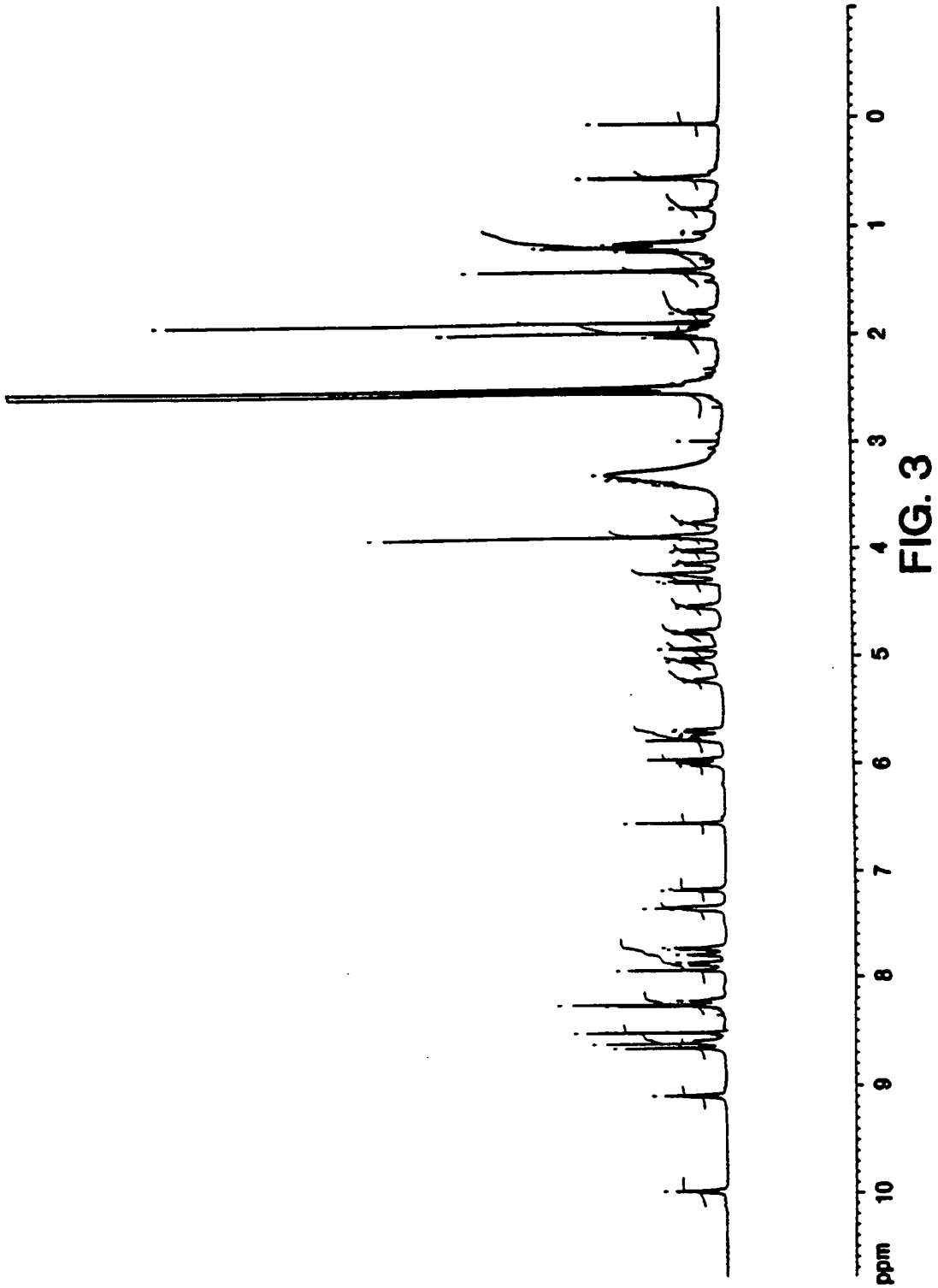



FIG. 3

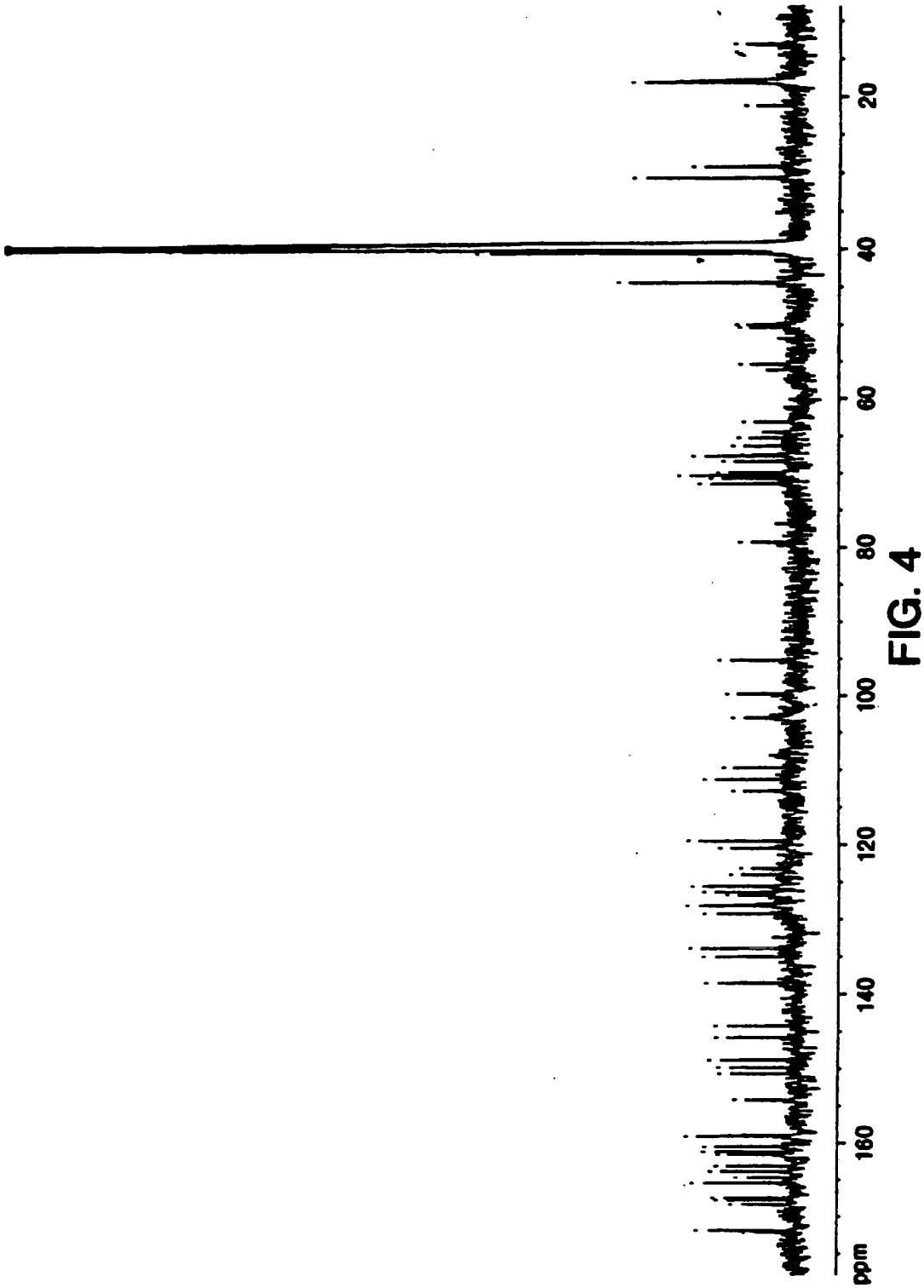
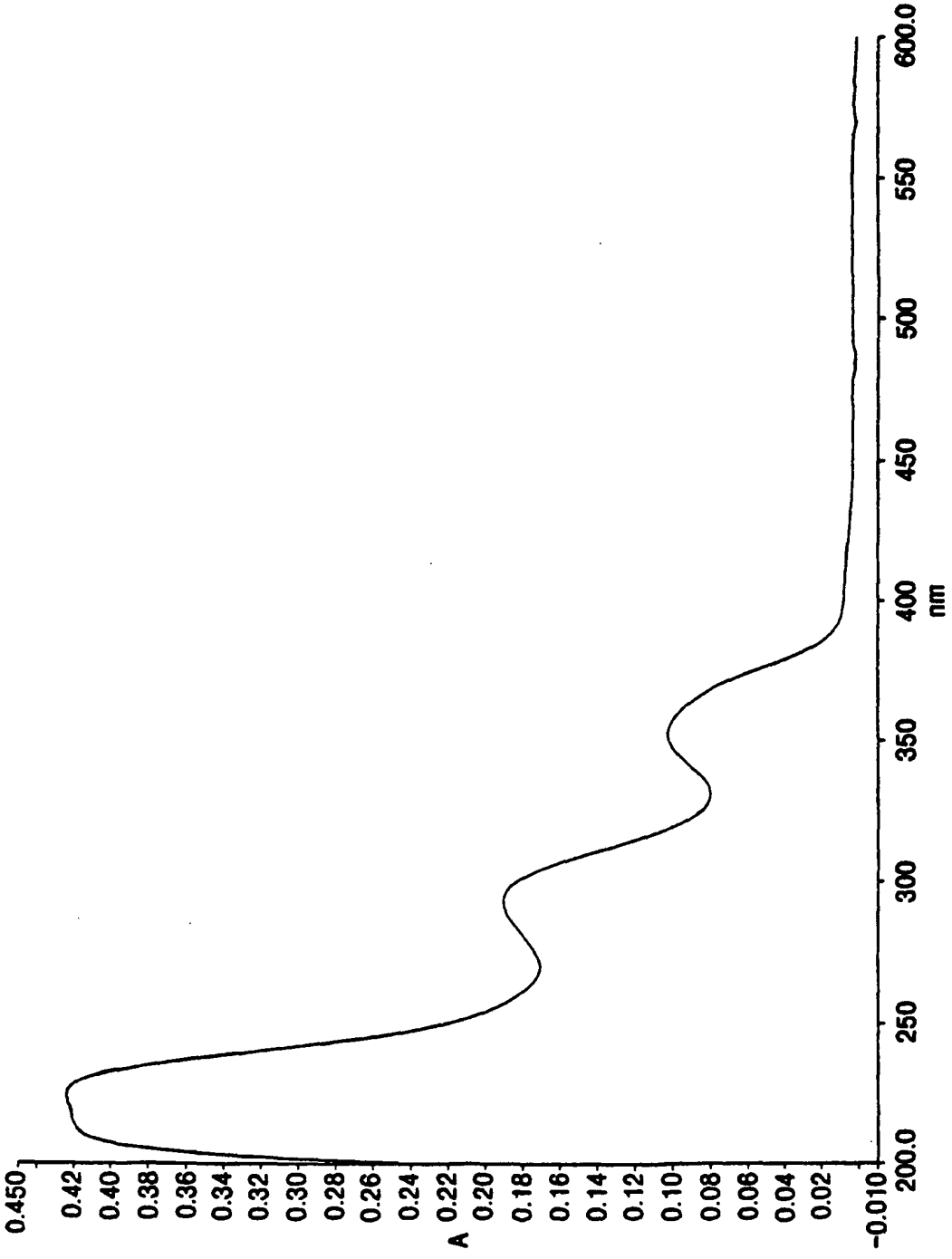



FIG. 4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0.18
0.16
0.14
0.12
0.10
0.08
0.06
0.04
0.02
-0.010
200.0
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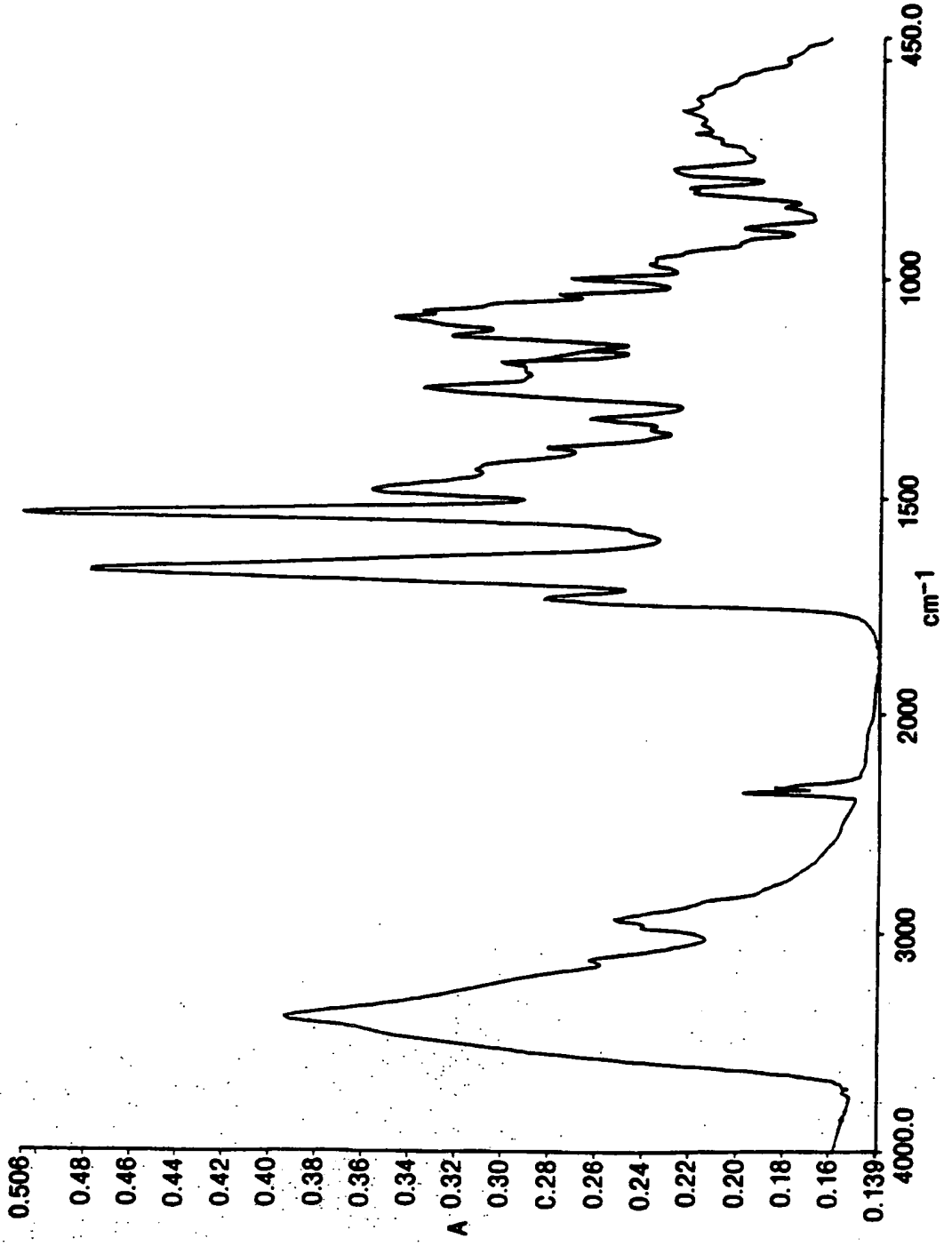
300
350
400
nm
450
500
550
600.0

FIG. 5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0.34
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0.26
0.24
0.22
0.20
0.18
0.16
0.139
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3000
2000
1500
1000
450.0
cm-1

FIG. 6

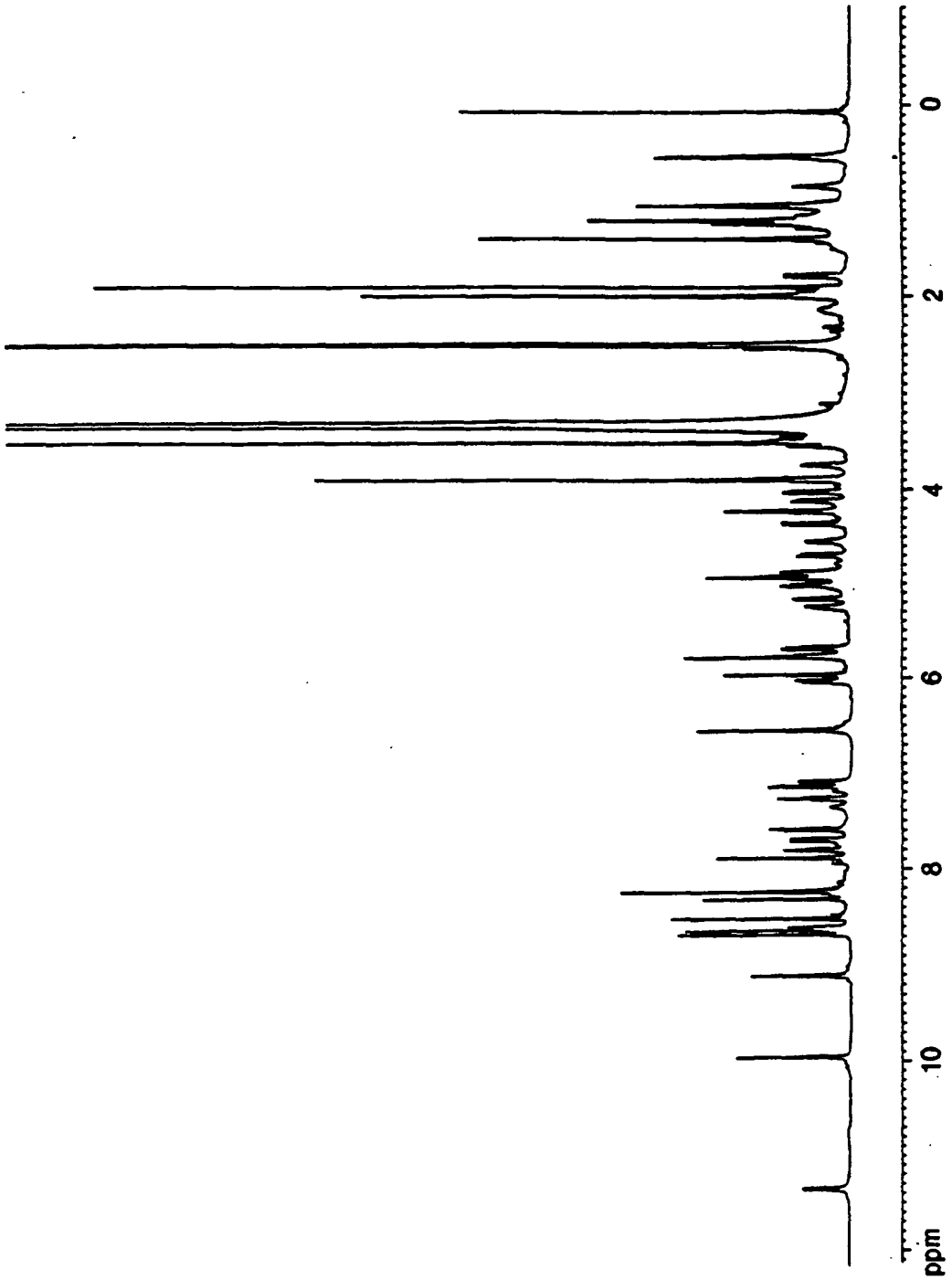
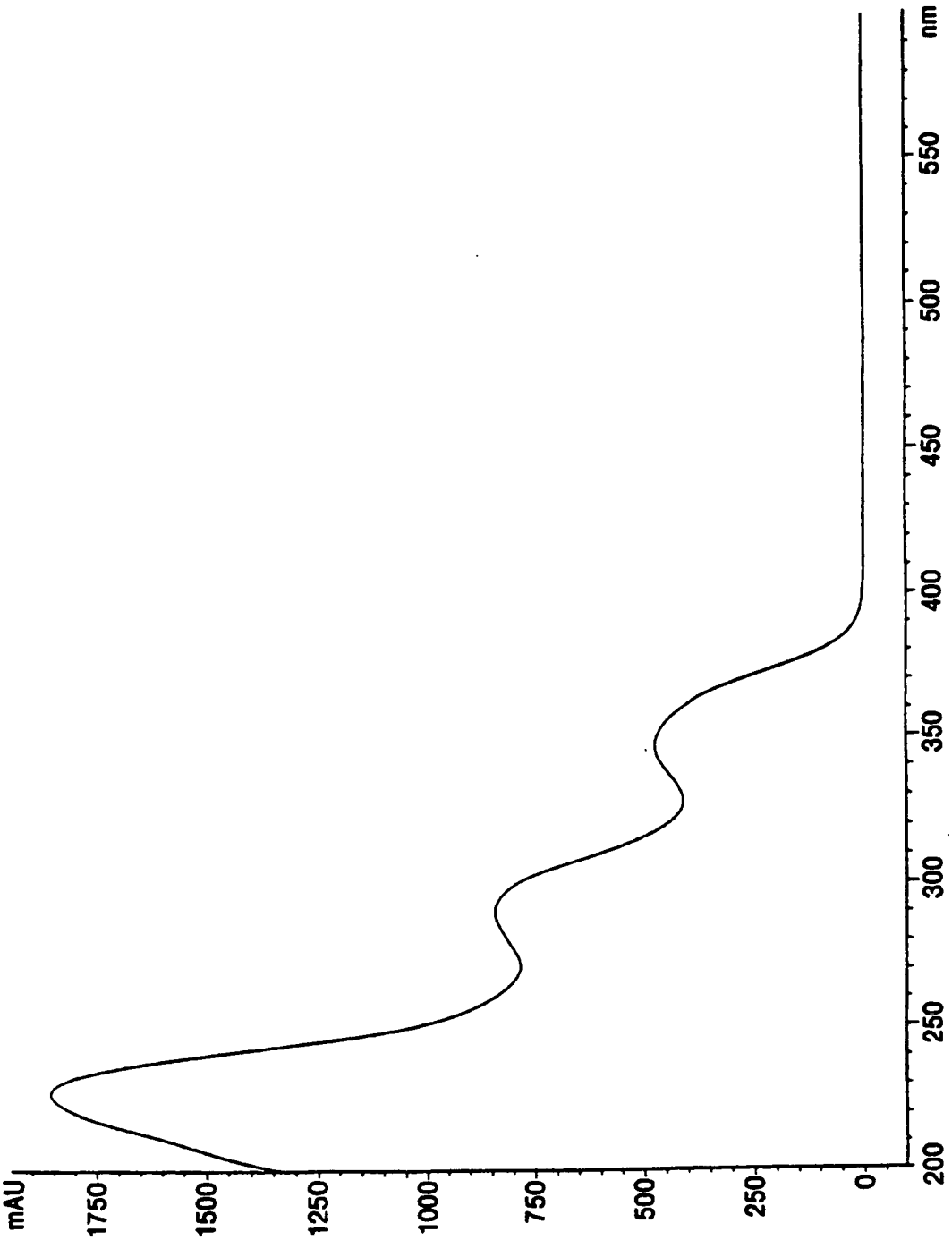



FIG. 7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750
500
250
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200
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300
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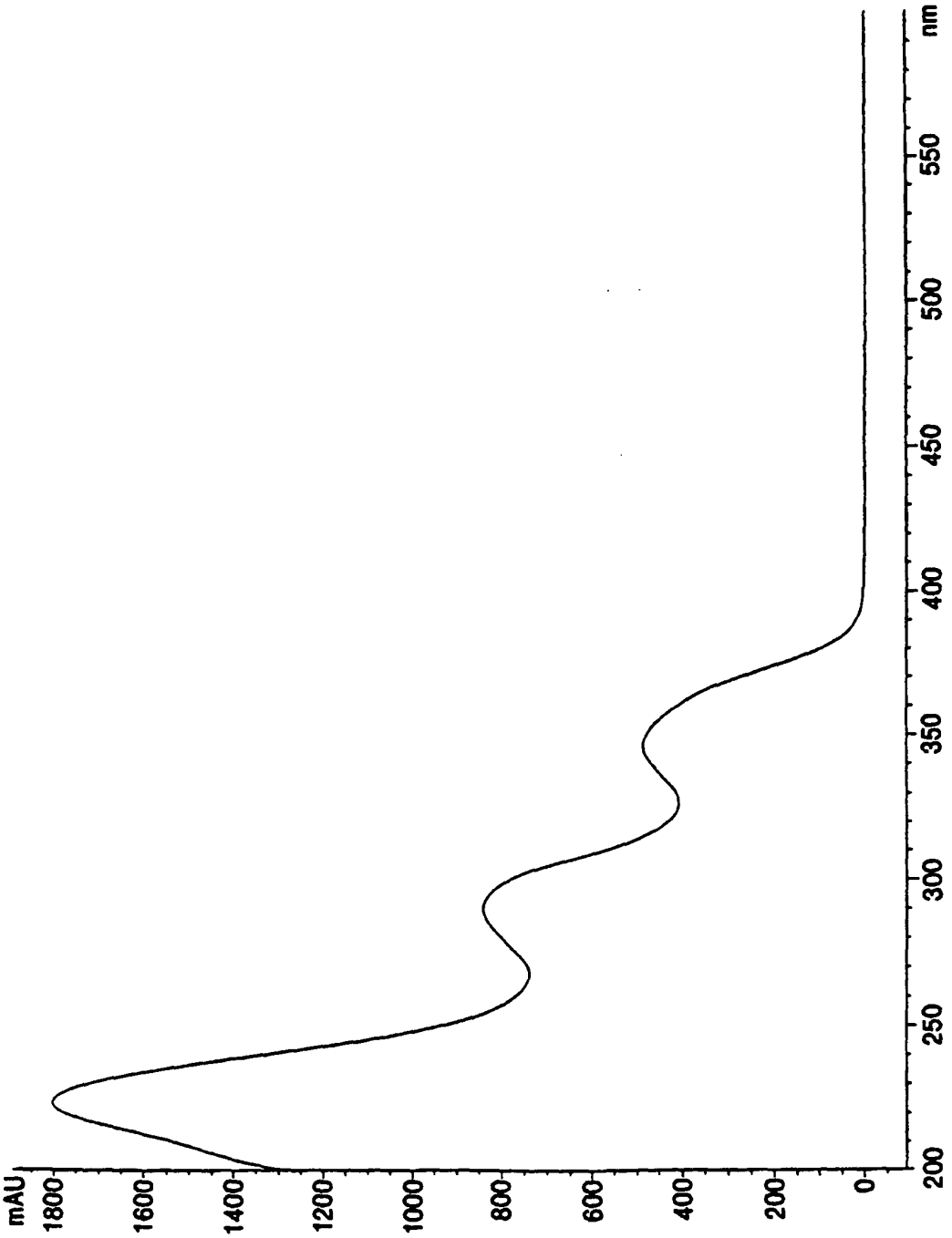
400
450
500
550
nm

FIG. 8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1400
1200
1000
800
600
400
200
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200
250
300
350
400
450
500
550
nm

FIG. 9

# INTERNATI NAL SEARCH REPORT

International application No.
PCT/US99/20242

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :C07H 17/08; A01N 43/04; C12P 19/56

US CL :536/7.1; 435/78, 119, 118, 117; 514/28

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/78, 119, 118, 117, 76; 514/279, 28, 183; 540/456; 536/7.1.

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Merck Index; Hackh's Chemical Dictionary

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

WEST, Derwent, CAS ONLINE, Medline, Biosis

Search terms: structure search, nocathiacin, Actinoplanes, Amycolata, antibiotic?, biotransform?, hydroxylat?

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| Y | US 4,478,831 A (KELLER-JUSLEN et al.) 23 October 1984, see entire document. | 1-12 |
| Y | US 5,451,581 A (LEE et al.) 19 September 1995, see entire document. | 1-12 |
| Y | NORTHCOTE et al. Glycothiohexide alpha, a Novel antibiotic produced by "Sebekia" sp. LL14E605. II Isolation and Physical-chemical characterization, The Journal of Antibiotics. August 1994, Vol. 47, No. 8, pages 894-900, see entire document. | 1-12 |

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

| | | | |
|---|---|---|---|
| * | Special categories of cited documents: | "T" | later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention |
| "A" | document defining the general state of the art which is not considered to be of particular relevance | "X" | document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone |
| "E" | earlier document published on or after the international filing date | "Y" | document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art |
| "L" | document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) | "&" | document member of the same patent family |
| "O" | document referring to an oral disclosure, use, exhibition or other means | | |
| "P" | document published prior to the international filing date but later than the priority date claimed | | |

| Date of the actual completion of the international search | Date of mailing of the international search report |
|---|---|
| 23 NOVEMBER 1999 | 13 DEC 1999 |
| Name and mailing address of the ISA/US<br>Commissioner of Patents and Trademarks<br>Box PCT<br>Washington, D.C. 20231<br>Facsimile No. (703) 305-3230 | Authorized officer<br>IRENE MARX<br>Telephone No. (703) 308-0196 |

Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/20242

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| Y | NORTHCOTE et al. Glycothiohexide alpha, a Novel antibiotic produced by "Sebekia" sp. LL14E605. III Structural Elucidation, The Journal of Antibiotics. August 1994, Vol. 47, No. 8, pages 901-908, see entire document. | 1-12 |
| Y | SASAKI et al. MJ347-81F4 A & B, Novel Antibiotics from Amycolatoposis sp.: Taxonomic Characteristics, Fermentation, and Antimicrobial Activity. The Journal of Antibiotics. August 1998, Vol. 51, No. 8, pages 715-721, see entire document. | 1-12 |
| Y | OKAZAKI et al. Taxonomy of Actinomycetes capable of hydroxylation of ML-236B (Compactin). The Journal of Antibiotics. September 1983, Vol. 36, pages 1176-1183, see entire document. | 9-12 |